

Chemical roadblocking of DNA transcription for nascent RNA display

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Materials Included:

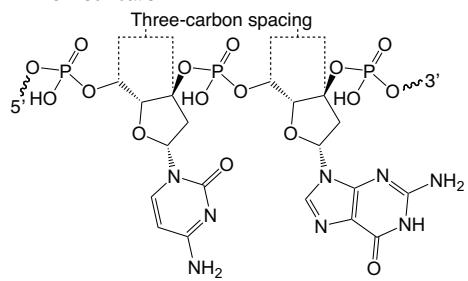
Figure S1. Chemical modifications shown in the context of DNA polynucleotides

Figure S2. Additional DNA template quality analyses

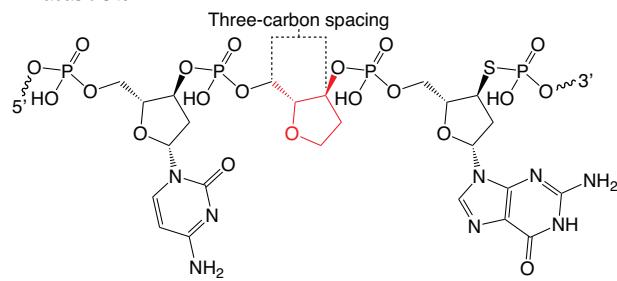
Figure S3. Internal etheno-dA DNA template QC replicate

Table S1. Oligonucleotides used for DNA template amplification

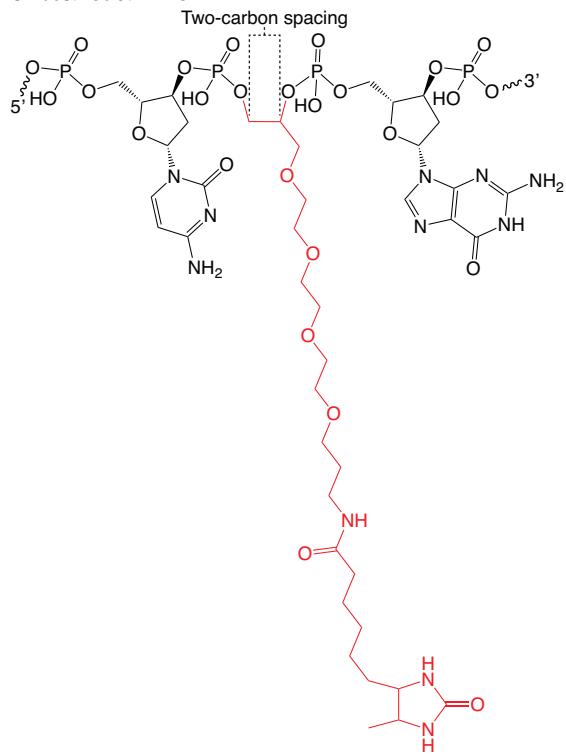
A no modification



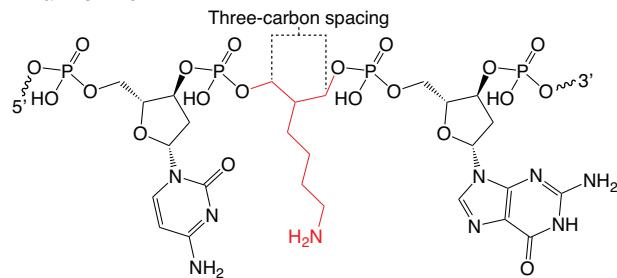
B abasic site



C desthiobiotin-TEG



D amino-linker



E etheno-dA

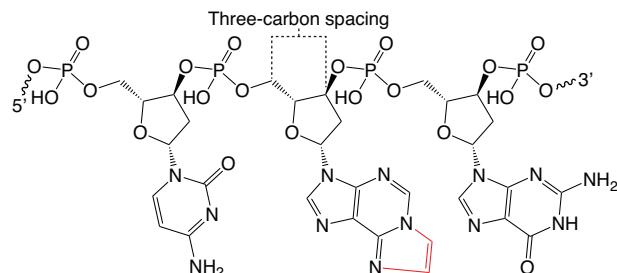


Figure S1. Chemical modifications shown in the context of DNA polynucleotides

An unmodified (A) dinucleotide is shown for comparison with abasic (B), desthiobiotin-TEG (C), amino-linker (D), and etheno-dA (E) lesions.

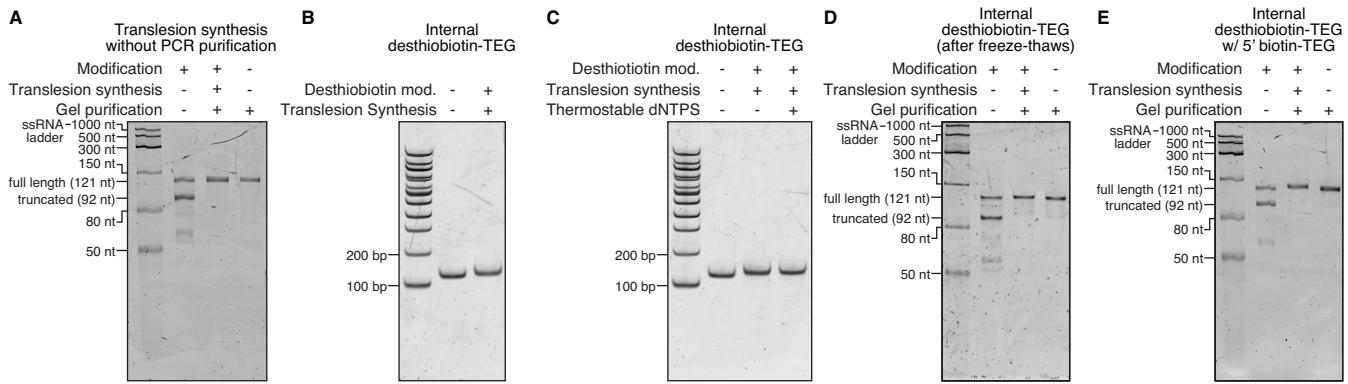


Figure S2. Additional DNA template quality analyses

(A) Denaturing PAGE quality analysis of DNA template with an internal desthiobiotin-TEG modification that was prepared by adding *Sulfolobus* Dpo4 directly to PCRs without an intermediate clean up step following amplification (compare to panel (E) and Figure 1E). The size marker is the Low Range ssRNA Ladder (New England Biolabs).

(B) Non-denaturing PAGE quality analysis of DNA template with an internal desthiobiotin-TEG modification. The size marker is the Quick-Load 100 bp DNA Ladder (New England Biolabs).

(C) Non-denaturing PAGE of DNA templates in which the initial PCR amplification was split to perform translesion synthesis with either standard dNTPs or a thermostable dNTP mixture in which dATP and dCTP were substituted with 2-amino-dATP and 5-propynyl-dCTP. The size marker is the Quick-Load 100 bp DNA Ladder (New England Biolabs).

(D) Denaturing PAGE quality analysis of DNA template with an internal desthiobiotin-TEG modification that was performed after several freeze-thaw cycles that occurred over the course of data collection. The size marker is the Low Range ssRNA Ladder (New England Biolabs).

(E) Denaturing PAGE quality analysis of DNA template with an internal desthiobiotin-TEG modification and a 5' biotin-TEG modification. The size marker is the Low Range ssRNA Ladder (New England Biolabs).

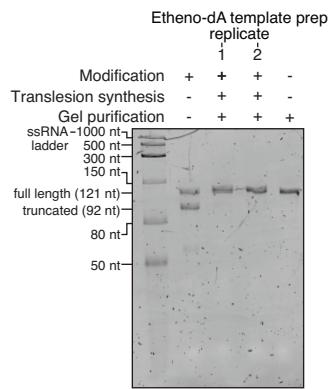


Figure S3. Internal etheno-dA DNA template QC replicate

Denaturing PAGE quality analysis of DNA templates with an internal etheno-dA. Two replicates of the etheno-dA template preparation are shown together for comparison. The size marker is the Low Range ssRNA Ladder (New England Biolabs).

Table S1. Oligonucleotides used for DNA template amplification

Below is a table of oligonucleotides used for the preparation of *in vitro* transcription DNA templates. The modification codes defined below are used for compatibility with the Integrated DNA Technologies ordering.

/5BiotinTEG/: 5' biotin-triethylene glycol
 /ideSBioTEG/: internal desthiobiotin-triethylene glycol
 /iUniAmM/: internal amino modifier
 /iEth-dA/: internal etheno-dA
 /5bioSG/: "standard" 5' biotin

Description	Sequence	Purification	ID
λP_R promoter	CTAACACCGTGCCTGTTGACTATTTACCTCTGGCGGTGATAATGGTTGCAT	HPLC	A
Biotinylated λP_R promoter	/5BiotinTEG/CTAACACCGTGCCTGTTGACTATTTACCTCTGGCGGTGATAATGGTTGCAT	HPLC	B
Reverse primer	AATGATACTGGCGACCACCGAGATCTACACGTTCAAGAGTTCTACAGTCCGACGATC	HPLC	C
Rev. primer w/ desthiobiotin-TEG	AATGATACTGGCGACCACCGAGATCTACAC/ideSBioTEG/GTTCAGAGTTCTACA GTCCGACGATC	HPLC	D
Rev. primer w/ amino-linker	AATGATACTGGCGACCACCGAGATCTACAC/iUniAmM/GTTCAGAGTTCTACAGTC CGACGATC	HPLC	E
Rev. primer w/ etheno-dA	AATGATACTGGCGACCACCGAGATCTACAC/iEth-dA/GTTCAGAGTTCTACAGTC CGACGATC	HPLC	F
Rev. primer w/ terminal biotin	/5biosG/AGATCTACACGTTCAAGAGTTCTACAGTCCGACGATC	HPLC	G
Template oligonucleotide	ACCTCTGGCGGTGATAATGGTTGCATATTAGATATTAGTCGATCGTCGGACTGTAG AACTCTGAAC	PAGE	H
Primer extension oligonucleotide	GTCGGACTGTAGAACTCTGAAC	None	I
Template for primer extension with +1C nt	AATGATACTGGCGACCACCGAGATCTACAGGTTCAAGAGTTCTACAGTCCGACGATC	HPLC	J
+1C template w/ desthiobiotin-TEG	AATGATACTGGCGACCACCGAGATCTACAG/ideSBioTEG/GTTCAGAGTTCTACA GTCCGACGATC	HPLC	K

The fully assembled DNA template sequence is shown below. Lowercase bases indicate the λP_R promoter.

Uppercase bases begin at the transcription start site and denote the transcribed region of the DNA template.

The U15 stall site is shown in red and the position of internal DNA modifications is denoted by [iMod].

Underlined sequence from the Illumina TruSeq Small RNA 3' Adapter.

Fully assembled DNA template:

ctaacacccgtgcgttgcactatttacctctggcggtgataatggttgcATATTAGATATTAGTCGATCGTCGGACTGTA
GAACCTCTGAAC [iMod] GTGTAGATCTCGGTGGTCGCCGTATCATT